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Determination of 3-MCPD in Some Edible Oils using GC-MS/MS

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THE presence of 3-monochloropropanediol (3-MCPD) in edible oils has been widely reported with its potential health risks. The aim of this study was to apply optimized recent updated and validated enhanced swift analytical indirect method for determining 3-MCPD in consumed edible oils (palm, palm olein, extra virgin olive, corn, sunflower, soybean, olive pomace) and blend of 5% sunflower oil with extra virgin olive oil, using selective and sensitive gas chromatography tandem triple quadrupole mass spectrometry (GC-MS/MS) employing deuterated 3-MCPD (3-MCPD-d5) as internal standard (IS) during the entire analytical procedure to obtain precise and accurate results. The occurrence and variation of 3-MCPD contents among the studied oils were found in different levels ranged from 93.1 µg/kg to 5634.1 µg/kg oil samples, with maximum value assigned for palm oil (5634.1 µg/kg) followed by palm olein (5576.8 µg/kg), corn oil (2447 µg/kg), sunflower oil (1817.3 µg/kg), soybean oil (1486.1 µg/kg), olive pomace oil (572.5 µg/kg), blend of 5% sunflower oil with extra virgin olive oil (210 µg/kg) and extra virgin olive oil (93.1 µg/kg). Palm, palm olein, corn, sunflower and soybean oils were found out of the limits recommended by the Commission Regulation (EU) 2020/1322, whereas, extra virgin olive oil, olive pomace oil and blend of 5% sunflower oil with extra virgin olive oil were in compliance and within the limits recommended by EU. Moreover, 3-MCPD content could be used as a good tool for authenticity and quality of genuine extra virgin olive oil.

Keywords: Edible oils; 3-Monochloropropanediol (3-MCPD); GC-MS/MS; Food contaminants

Introduction

3-Monochloropropanediol (3-MCPD) is the most toxic food contaminant formed during thermal processing of refined edible oils. Refining of edible oils at a high temperature generates byproducts such as 3-MCPD [1, 2]. 3-MCPD with molecular formula of $C_3H_7ClO_2$, molar mass of 110.539 g mol^{-1}, density of 1.32 g cm^{-3}, and boiling point of 213 ^oC exists either in free form, or in bound form called 3-MCPD esters in different foodstuffs that has passed through cooking processes using cooking oil. In order to produce safe edible oils, usage of new materials and methods in refining process are explored, and the effects of byproducts generated during this process on human body are closely monitored [3]. Deodorization is a key step during oil refining for overall oil quality, especially the deodorization temperature is the direct and critical parameter [4]. The UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment [5] have considered the carcinogenicity of 3-MCPD and its fatty acid esters. European Commission [6], the regulatory arm of the European Union, namely the European Commission (EC) Scientific Committee on Food (SCF), adopted the tolerable daily intake (TDI) of 2 µg/kg body weight (bw) for 3-MCPD.

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Furthermore, The Joint WHO/FAO Expert Committee on Food Additives (JECFA) also recommended a provisional maximum TDI of 2 µg/kg bw for 3-MCPD [7, 8]. In March 2016, the European Food Safety Authority (EFSA) issued an extensive report warning about the possible health consequences of contaminants created during the processing of edible oils [9, 10]. EFSA specifically identified 3-MCPD, which was classified as a possible human carcinogen by International Agency for Research on Cancer (IARC) and documented in IARC Monographs [11]. Finally, there is a growing concern associated with the release of bound 3-MCPD into its free form that results in bioavailability and absorption of 3-MCPD into human body fluids and tissues, e.g. human breast milk, which requires additional studies [12-14].

Kidney is one of the primary target organs for 3-MCPD [15] with induced renal injury [16], toxicity in kidney, lung, testis, and heart [17-19], immunosuppressive activity [20], toxic effects on male reproduction [21] and is regarded as a rat carcinogen inducing testicular lesions, Leydig-cell and mammary gland tumors in males and kidney tumors in both genders [22, 23]. Combination of histopathological examination, clinical chemistry and metabolomics analyses resulted in systematic comprehensive assessment of the long-term toxicity of 3-MCPD, where renal tubular hyaline cast accumulation with epithelium cell degeneration and potential kidney toxicity were major histopathological changes with thin appearance and subdued behavior accompanied by decreases in bw. Microscopy revealed tubular basophilia in kidneys, exfoliated degenerative germ cells in the lumen of the seminiferous tubule of testes, vacuolation in the brain, axonal degeneration of sciatic nerve and cardiomyopathy [24, 25].

Current analytical methods for 3-MCPD determination still need to be improved regarding sample preparation. The methods based on liquid extraction should be replaced with more efficient and environment-friendly. Currently, the widely applied method developed by DGF is time-consuming and requires the use of significant amounts of solvents. Undoubtedly, the methods should be as cheap and simple as possible in order to apply them in industrial laboratories for routine analyses of complex matrices of foodstuffs. Moreover, the formation routes and mechanisms need to be fully explained in relation to industrial processing, with the aim of mitigating the presence of 3-MCPD in foodstuffs by changing the process conditions, as in oil refining process [26-29] or by treating the already processed product, as oils after refining process on adsorbent material or with enzymes [30, 31].

Accurate, rapid, and high-sensitivity chemical analytical methods are needed for detecting the presence and levels of 3-MCPD esters in different types of food. To date, the reported analytical methods for 3-MCPD esters can be divided into indirect and direct detection approaches. The indirect method involves releasing the free 3-MCPD from all the fatty acid esters of 3-MCPD in the sample, preparing derivatives for analysis, and quantifying the amount of free 3-MCPD, with the total amount of 3-MCPD esters to be reported as the equimolar of free 3-MCPD contents. On the other hand, the direct approach characterizes and quantifies each individual 3-MCPD ester in food samples directly, which is straightforward and easy to understand but much more difficult in sample purification and method development during practical utilizations compared to that for the indirect approach [32].

Commission Regulation (EU) 2020/1322 of 23 September 2020 amending Regulation (EC) No. 1881/2006 as regards maximum levels of 3-monochloropropanediol (3-MCPD), 3-MCPD fatty acid esters and glycidyl fatty acid esters in certain foods, recommended the following in "*Section 4: 3-monochloropropanediol (3-MCPD), 3-MCPD fatty acid esters and glycidyl fatty acid esters"* [33].

4.3.1. Vegetable oils and fats, fish oils and oils from other marine organisms placed on the market for the final consumer or for use as an ingredient in food falling within the following categories, with the exception of the foods referred to in 4.3.2 and of virgin olive oils, the maximum level is 1250 µg/kg of 3-MCPD.

- oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils (composed of refined olive oil and virgin olive oil) and mixtures of oils and fats with oils and fats only from this category; the maximum level is 1250 µg/kg of 3-MCPD.
- other vegetable oils (including pomace olive oils), fish oils and oils from other marine organisms and mixtures of oils and fats with oils and fats only from this category; the maximum level is 2500 µg/kg of 3-MCPD.

4.3.2. Vegetable oils and fats, fish oils and oils from other marine organisms destined for the production of baby food and processed cereal-based food for infants and young children, the maximum level is 750 µg/kg of 3-MCPD.

The aim of this study was to investigate the occurrence of 3-MCPD in some edible oils using recent optimized validated indirect method of determination and comparison of 3-MCPD contents among different edible oils and their compliance with standard regulations recommended by Commission Regulation (EU) 2020/1322 [33].

Materials and Methods

Materials

Edible oils from various plant origins were purchased from local markets and used in the study. Palm oil, palm olein oil, extra virgin olive oil (EVOO), corn oil, sunflower oil, soybean oil, olive pomace oil and blend of EVOO + 5% sunflower oil.

Chemicals and reagents

All solvents and chemicals were of Analytical and HPLC grades obtained from Sigma Chemical Co. (USA). Sodium methoxide, glacial acetic acid, acetone, diethyl ether, ethyl acetate, *t*-butyl methyl ether, *n*-hexane, phenylboronic acid (PBA), 3-MCPD and 3-MCPD-d5 were purchased from Sigma Chemical Co. (USA), while 3-MCPD-1,2-dipalmitoyl ester and 3-MCPD-1,2-dipalmitoyl ester-d5 were purchased from Toronto Research Chemicals Inc. (Canada).

Physicochemical quality parameters and fatty acid composition

Refractive index (RI), and peroxide value (PV) expressed as milliequivalent of O_2/kg oil, were determined according to AOAC [34]. Acidity or free fatty acid (FFA) expressed as oleic acid %, was determined according to ISO 660:2020 [35]. Conjugated constituents were determined by measuring UV absorption at 232 nm for diene and 270 nm for triene in purified solvent according to ISO 3656:2011 [36]. Fatty acid composition was converted into methyl ester and determined by GC according to ISO 12966-2:2017 [37].

Determination of 3-MCPD

3-MCPD contents in edible oils samples under study were determined according to the analytical procedures described by Aboelhassan *et al*. [38], where an enhanced swift analytical method for the determination of bound 3-MCPD has been optimized. The method enhancements include shorter sample preparation time, a rapid run program in triple quadrupole GC-MS/MS, besides, augmented selectivity and sensitivity. It has been verified for fitness-for-use in terms of selectivity, sensitivity, accuracy, range and measurement uncertainty for compliance with the international performance requirements, the limit of quantification (LOQ) and the limit of detection (LOD) were estimated to be less than 2 and 0.5 µg/kg, respectively. The recoveries at different spiked levels ranged from 98 to 106%. The reproducibility (expressed as relative standard deviation RSD) was less than 8% while measurement uncertainty was in the range of ±18% [38].

Standard solutions preparation

100 µg/mL stock solutions of 3-MCPD and 3-MCPD-d5 were prepared by their individual dissolving in deionized water and stored in the refrigerator. 1 µg/mL solutions of 3-MCPD and 3-MCPD-d5 were prepared by diluting appropriate volumes in deionized water and stored in the refrigerator. Calibration standard solutions with concentrations of 1, 5, 10, 20 and 50 µg/L were prepared by diluting 1 µg/mL 3-MCPD standard working solution with deionized water. 100 and 132 µg/mL standard solutions of 3-MCPD-1,2-dipalmitoyl ester and 3-MCPD-1,2-dipalmitoyl ester-d5, respectively, were prepared by dissolving both of them separately in 25 mL of *tert*-butyl methyl ether and stored in the refrigerator. 1 and 1.3 µg/mL standard solutions of 3-MCPD-1,2-dipalmitoyl ester and 3-MCPD-1,2-dipalmitoyl ester-d5, respectively, were prepared by diluting appropriate volumes in *tert*-butyl methyl ether and stored in the refrigerator.

Calibration standards derivatization for standard curves

An exact volume of 2 mL was taken separately from each 3-MCPD calibration standard solution into 4 mL capped vials. A 40 µL aliquot of 3-MCPD-d5 (1 µg/mL) was added as an internal standard, followed by the addition of 400 µL of 20% PBA. The mixture was well shaken for 1 min, transferred into a water bath, kept at 85°C for 20 min and left to cool at room temperature. A 2 mL aliquot of *n*-hexane was added, and the mixture was well shaken for 1 min. *n*-Hexane layer was filtered through a 0.45 µm membrane filter before injection into triple quadrupole GC-MS/MS.

Sample preparation

For 3-MCPD extraction, 2 ± 0.1 g of sample (oil) was weighed in a 50 mL centrifuge tube, 777 µL of 1.3 µg/mL 3-MCPD-1,2-dipalmitoyl ester-d5 was added as an internal standard, then, 8 mL of diethyl ether was added. The mixture was well shaken for 2 min. followed by addition of 200 µL of 2M sodium methoxide. After 1-2 min., 100 µL of glacial acetic acid was added, then the mixture was added to 10 mL of deionized water added prior to a tube, with well shaking for 2 min., followed by centrifugation at 4000 rpm for 5 min., then 2 mL of aqueous layer was taken into a 4 mL capped vial and 400 µL of 20% PBA was added. The vial was put into a water bath at 85°C for 20 min. It was left to cool, then 2 mL of *n*-hexane was added, followed by well shaking for 1 min., and then *n*-hexane layer was filtered through a 0.45 µm pore size membrane before injection.

Instrumentation

Chromatographic separation and mass spectrometric determination techniques were applied using a gas chromatography tandem triple quadrupole mass spectrometry system (7890B Triple Quadrupole GC-MS/MS - Agilent Technologies, USA).

Inlet system was split/splitless with inlet deactivated (inert) liner (Agilent Technologies, USA) with an Agilent Column J&W Ultra Inert DB-35MS GC column (20.0 m length, 0.18 mm internal diameter and 0.18 μm film thicknesses).

For multiple reaction monitoring transitions; 2 MRM transitions were used (2 precursor ions and 2 product ions), one for quantification ([a]) and the other for qualification ([b]). MRM details are represented in **Table 1.**

Table 1. Multiple reaction monitoring transitions

Analyte	Precursor ion, *m/z*	Product ion, *m/z*	Dwell time, ms	Collision energy, eV
3-MCPD[a]	196	147	80	20
3-MCPD[b]	198	147	80	20
3-MCPD-d5[a]	203	150	80	20
3-MCPD-d5[b]	201	150	80	20

[a]for quantification, [b]for qualification

Results and Discussion

Physicochemical quality parameters

Physicochemical quality parameters of the studied oils were listed in **Table 2**.

Refractive index plays an important role in characterizing oils, it has relationship to structure. The obtained refractive indices of the studied oils; PO, POO, EVOO, CO, SO, SPO, OPO and EVOO+5%SO were 1.4550, 1.4580, 1.4678, 1.4720, 1.4727, 1.4725, 1.4726 and 1.4685, respectively. These variations in refractive indices of the corresponding oils under investigation were attributed to their structure, chain length and the differences in fatty acid composition of these oils containing different profiles, especially linoleic acid ($C_{18:2}$) content. Peroxide value gives the initial evidence of rancidity in unsaturated fats and oils. It gives a measure of the extent to which an oil sample has undergone primary oxidation. All investigated oils in the study showed a low peroxide values as illustrated in **Table 2.** The low peroxide values indicated the high initial quality and freshness of the studied oils. Also, the amounts of free fatty acids were found generally too small. The observed low acidity indicated that the oils did not undergo hydrolytic processes and may have a long shelf life emphasis the high quality of oils. It was ranged from 0.04 to 0.59% in the studied oils. Acidity is a measure of the amount of free fatty acids (FFA) present in oil due to both hydrolysis of its triglycerides and oxidation of double bonds of the unsaturated acyl chains which produced free fatty acids with low molecular weight. It has been frequently used as an important parameter to monitor quality of oils and to show the case of hydrolysis and oxidation induced in the oil. FFA content increases with hydrolysis of triacylglycerols (TAGs) and diacylglycerols (DAGs) [39]. FFA (or possibly free hydrogen chloride) is necessary for formation of 3-MCPD ester which is formed 2–5 times faster from DAGs than from monoacylglycerols (MAGs) or TAGs [40, 41]. Absorbencies at 232 nm of PO, POO, EVOO, CO, SO, SPO, OPO and EVOO+5%SO were 1.142, 1.022, 1.399, 1.690, 1.891, 1.661, 1.88 and 1.487, respectively. Whereas, absorbencies at 270 nm of PO, POO, EVOO, CO, SO, SPO, OPO and EVOO+5%SO were 0.165, 0.132, 0.134, 0.304, 0.284, 0.237, 0.281 and 0.180, respectively. Specific extinction is a quality parameter that provides information about oxidative state of oils and can aid in detection of fraud [42]. The initial characteristics of vegetable oils used in this study indicated that all the oils were of good quality [43-49].

Table 2. Physicochemical quality parameters of the studied oils

Parameter	PO	POO	EVOO	CO	SO	SBO	OPO	EVOO+5% SO
RI	1.4550 40 °C	1.4580 40 °C	1.4678 25 °C	1.4720 25 °C	1.4727 25 °C	1.4725 25 °C	1.4726 25 °C	1.4685 25 °C
FFA (oleic acid %)	0.37	0.59	0.29	0.08	0.06	0.04	0.05	0.26
PV [meq O_2/kg oil]	1.80	1.01	3.86	0.87	1.05	0.77	1.08	1.05
UV Abs_{232} (Diene)	1.142	1.022	1.399	1.690	1.891	1.661	1.88	1.487
UV Abs_{270} (Triene)	0.165	0.132	0.134	0.304	0.284	0.237	0.281	0.180

PO: Palm oil, POO: Palm olein oil, EVOO: Extra virgin olive oil, CO: Corn oil, SO: Sunflower oil, SBO: Soybean oil, OPO: Olive pomace oil

Fatty acid composition

. **Table 3. Fatty acid composition (%) of the studied oils**

Fatty Acid	PO	POO	EVOO	CO	SO	SBO	OPO	EVOO+5% SO
$C_{8:0}$	0.029	0.15	*ND	*ND	*ND	*ND	*ND	*ND
$C_{10:0}$	0.023	0.17	*ND	*ND	*ND	*ND	*ND	*ND
$C_{12:0}$	0.241	0.19	*ND	*ND	*ND	*ND	*ND	*ND
$C_{14:0}$	1.09	0.98	0.06	0.08	0.13	0.07	0.01	0.06
$C_{16:0}$	42.08	39.06	15.36	10.97	6.86	9.88	15.2	13.36
$C_{16:1}$	0.166	0.22	1.22	0.09	0.10	0.09	1.21	1.10
$C_{17:0}$	0.10	0.09	0.06	0.06	0.03	0.09	0.06	0.05
$C_{17:1}$	ND	ND	0.09	0.04	0.02	0.05	0.1	0.07
$C_{18:0}$	4.33	4.16	2.34	1.96	3.40	4.08	2.41	2.50
$C_{18:1}$	41.25	43.44	70.03	28.66	27.14	24.31	70.08	67.96
$C_{18:2}$	9.95	10.60	8.88	55.67	60.77	54.49	8.94	12.46
$C_{18:3}$	0.189	0.24	0.90	0.76	0.19	5.25	1.0	0.47
$C_{20:0}$	0.34	0.37	0.47	0.38	0.29	0.35	0.48	0.31
$C_{20:1}$	0.126	0.16	0.36	0.24	0.16	0.18	0.36	0.28
$C_{22:0}$	*ND	0.06	0.12	0.20	0.67	0.43	0.14	0.39
Σ SFA	48.359	45.39	18.41	13.65	11.38	14.90	18.3	17.77
Σ USFA	51.515	54.44	81.49	85.46	88.59	85.08	81.69	81.24

*ND: Not Detected, PO: Palm oil, POO: Palm olein oil, EVOO: Extra virgin olive oil, CO: Corn oil, SO: Sunflower oil, SBO: Soybean oil, OPO: Olive pomace oil, SFA: Saturated fatty acids, USFA: Unsaturated fatty acids

Fatty acid compositions (%) of the studied oils were shown in **Table 3**. All the studied oils had elevated amounts of total unsaturated fatty acids (Σ USFA) ranged from 88.59% in SO to 51.515% in PO. Whereas, total saturated fatty acids (Σ SFA) ranged from 48.359% in PO to 11.38% in SO. From the composition point of view for unsaturated fatty acids; oleic acid ($C_{18:1}$) was the major in EVOO > OPO > EVOO+5%SO > POO > PO > CO > SO > SBO, whereas, linoleic acid ($C_{18:2}$) was the major in SO > CO > SBO > POO > PO > EVOO+5%SO > OPO > EVOO and linolenic acid ($C_{18:3}$) was the major in SBO > OPO > EVOO > CO > EVOO+5%SO > POO > SO > PO. From the composition point of view for saturated fatty acids; palmitic acid ($C_{16:0}$) was the major in PO > POO > EVOO > OPO > EVOO+5%SO > CO > SBO > SO, whereas, stearic acid ($C_{18:0}$) was the major in PO > POO > SBO > SO > EVOO+5%SO > OPO > EVOO > CO and myristic acid ($C_{14:0}$) was the major in PO > POO with traces in the remaining oils. The obtained results for fatty acid composition of the studied oils were in their standard range [43, 45, 46, 49, 50].

Determination of 3-MCPD

The calibration curve of 3-MCPD was shown in **Figure (1)**. The results of calibration curve revealed that there was a linear relationship between different concentrations of 3-MCPD with R^2 being 0.99989356.

3-MCPD contents (µg/kg) in the studied oils were shown in **Table 4 and Figures (2-9).**

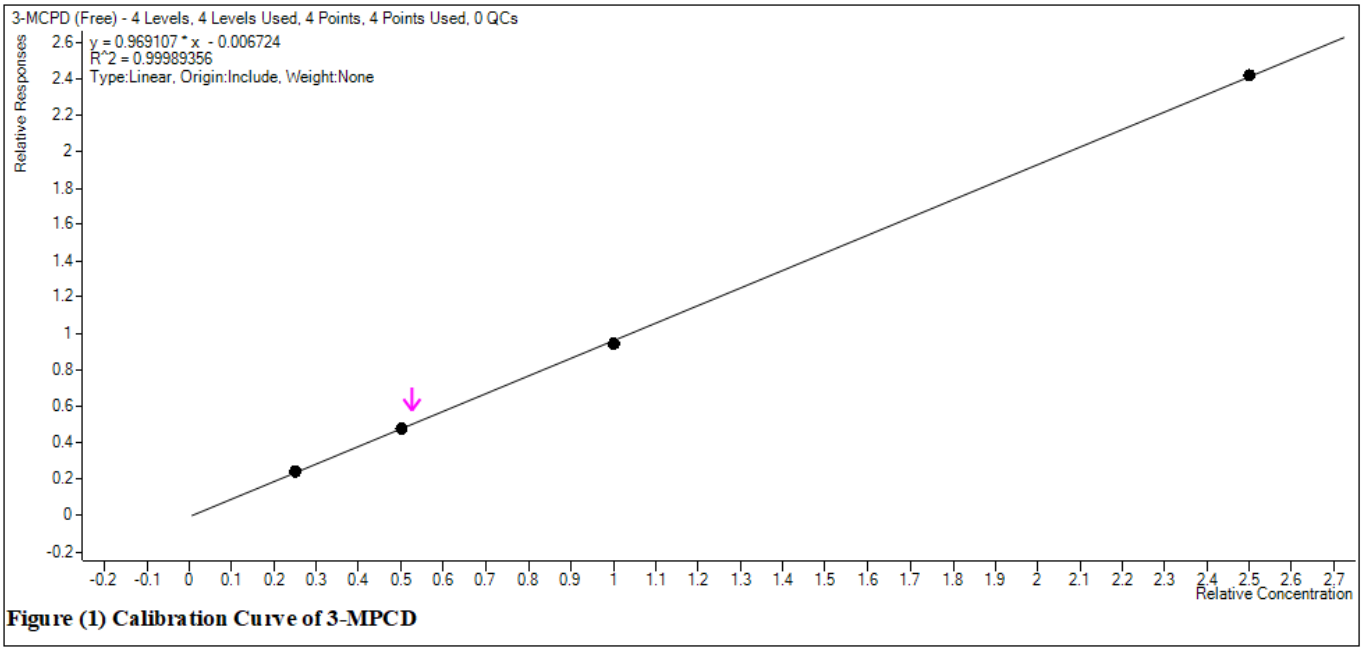


Figure (1) Calibration Curve of 3-MPCD

Table 4. Contents of 3-MCPD (µg/kg) in the studied oils

Edible oil	3-MCPD (µg/kg)	3-MCPD Maximum level (µg/kg) recommended by Commission Regulation (EU) 2020/1322
PO	5634.1	1250
POO	5576.8	1250
EVOO	93.1	1250
CO	2447.0	1250
SO	1817.3	1250
SBO	1486.1	1250
OPO	572.5	2500
EVOO+ 5% SO	210.0	1250

PO: Palm oil, POO: Palm olein oil, EVOO: Extra virgin olive oil, CO: Corn oil, SO: Sunflower oil, SBO: Soybean oil, OPO: Olive pomace oil

Results revealed the occurrence and variation in 3-MCPD content among the studied oil samples with different levels ranged from 93.1 µg/kg to 5634.1 µg/kg , with maximum value assigned for PO (5634.1 µg/kg) followed by POO (5576.8 µg/kg), CO (2447 µg/kg), SO (1817.3 µg/kg), SBO (1486.1 µg/kg), OPO (572.5 µg/kg), EVOO+5% SO (210 µg/kg) and EVOO (93.1 µg/kg). Palm, palm olein,

corn, sunflower and soybean oils were found out of the limits recommended by Commission Regulation (EU) 2020/1322, whereas, extra virgin olive, olive pomace and blend of 5% sunflower with extra virgin olive oils were found within the recommended limit by Commission Regulation (EU) 2020/1322 [33].

3-MCPD contents detected in oils were ordered from the highest to lowest as follows: PO > POO > CO > SO > SBO > OPO > EVOO+5%SO > EVOO. The available data have strongly suggested that processing affects the overall 3-MCPD ester levels in oils and foods. Refined commercial olive, sunflower and soybean oils have relatively greater (3–32 times) concentrations of 3-MCPD esters than their unrefined counterparts [32].

The obtained results agreed with those reported by WHO: Concentrations of 3-MCPD esters in refined oils increase incrementally as follows: soybean oil < sunflower oil < palm oil [8].

OPO which comes from poorer starting raw materials had much higher content of 3-MCPD than EVOO. It is probably due to the use of high temperatures during both the drying process, which has to be carried out before the solvent extraction step, and during the deodorization step in refining process. This is useful in considering 3-MCPD content as an analytical tool and complementary indicator of adulteration in EVOO where refined oils have been added [42, 51], and it may be used as a good tool for authenticity, identity and quality parameters of EVOO with many methods such as UV-visible, FTIR spectroscopy, ^{1}HNMR, DSC [52-56].

PO and POO have highest 3-MPCD contents which are more than double of CO and more than triple of SO and SBO, agreed with Hew et al. [57].

The mechanism for the formation of 3-MCPD esters and palm oil related compounds is assumed that glycerol, MAGs, DAGs and phospholipids are precursors on the way to the esters. The formation of free 3-MCPD strongly depends on temperature and the content of lipids, glycerol, salt and water. Additionally to triacylglycerols (TAGs), fats and oils contain varying amounts of free fatty acids, MAGs and DAGs depending on the history of the raw material before processing. While oils like rapeseed, sunflower, olive or soybean oil contain between 1 and 3% DAGs, in palm oil amounts between 6 and 10% can be found resulting from the activity of lipases after maturation before inactivation. Crude coconut, palm and palm kernel oils are distinguished by high amounts of free fatty acids up to 7%, while the other oils only contain between 1 and 2% [58].

Following the study of factors impacting the formation of 3-MCPD during palm oil production, a root-cause analysis was performed in order to map the parameters potentially responsible for the occurrence of MCPD diesters in refined palm oil and related fractions [59] shown in **Figure (10).**

Chlorine-containing compounds exist in the form of either inorganic or organic. Inorganic chlorine salts of calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), iron (III) chloride ($FeCl_2$) and iron (III) chloride ($FeCl_3$) originate from fertilizers and irrigation process. $FeCl_2$ and $FeCl_3$ were reported to have higher contents compared to other sources of inorganic chlorine. The occurrence of organochlorines in crude palm oil (CPO) prior to processing indicates that the chlorinated compounds might be present in the oil palm fruitlets even before harvesting. The correlations between total chlorine content in vegetable oil and 3-MCPD level have also been established by many researchers. It was suggested that organochlorines might indirectly act as a chlorine donor during oil deodorization process, which takes place at a temperature above 180 ^{o}C. During the process, the sum of organochlorines depleted as the sum of 3-MCPD diesters increased and HCl was formed. Therefore, HCl is suspected to be one of the reactive compounds contributing to the formation of 3-MCPD. It has also been identified that the 3-MCPD ester level increases with the addition of ionic bound chlorine, for instance tetrabutylammonium chloride (TBAC) during the modeling of deodorization for vegetable oil. A similar finding, involving a laboratory scale of CPO physical refining highlighted the potential of natural organochlorine as a chlorine precursor due to its oil solubility, which inhibits its removal during rinsing step with water. Conventionally, among fertilizers used in the oil palm plantation are ammonium chloride, NH_4Cl and potassium chloride, KCl. In addition, the herbicides used in the plantation, namely diuron,2,4-D amine, dicamba and fluroxypyr also contained chlorine compound. As most of the herbicides used in the plantation are water soluble, the oil palm fruitlet is likely to be exposed to the chlorine compound through nutrient uptake by the palm trees and through leaching process, where the chlorine compound dissolved in groundwater which will then be absorbed by the palm trees during cultivation. The irrigation water used in the oil palm plantations is also a possible source of chlorine precursor, in addition to the treated wastewater from the treatment facilities that used $FeCl_3$ as flocculants. Moreover, the bruising of fresh fruit bunches (FFBs) was identified to correspond with the increase in FFA content in the palm oil during harvesting and transportation to the mills. The FFA formation is equivalent to the formation of diacylglycerols (DAGs) and monocylglycerols (MAGs), which influence the formation of 3-MCPD [60, 61].

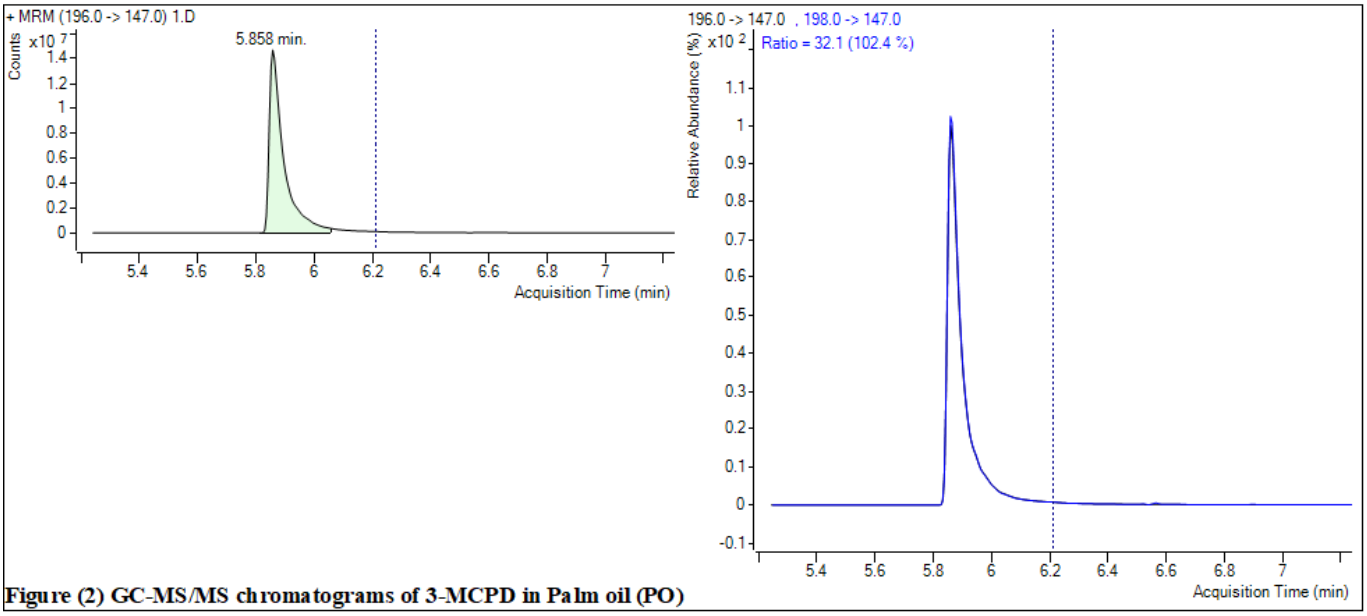


Figure (2) GC-MS/MS chromatograms of 3-MCPD in Palm oil (PO)

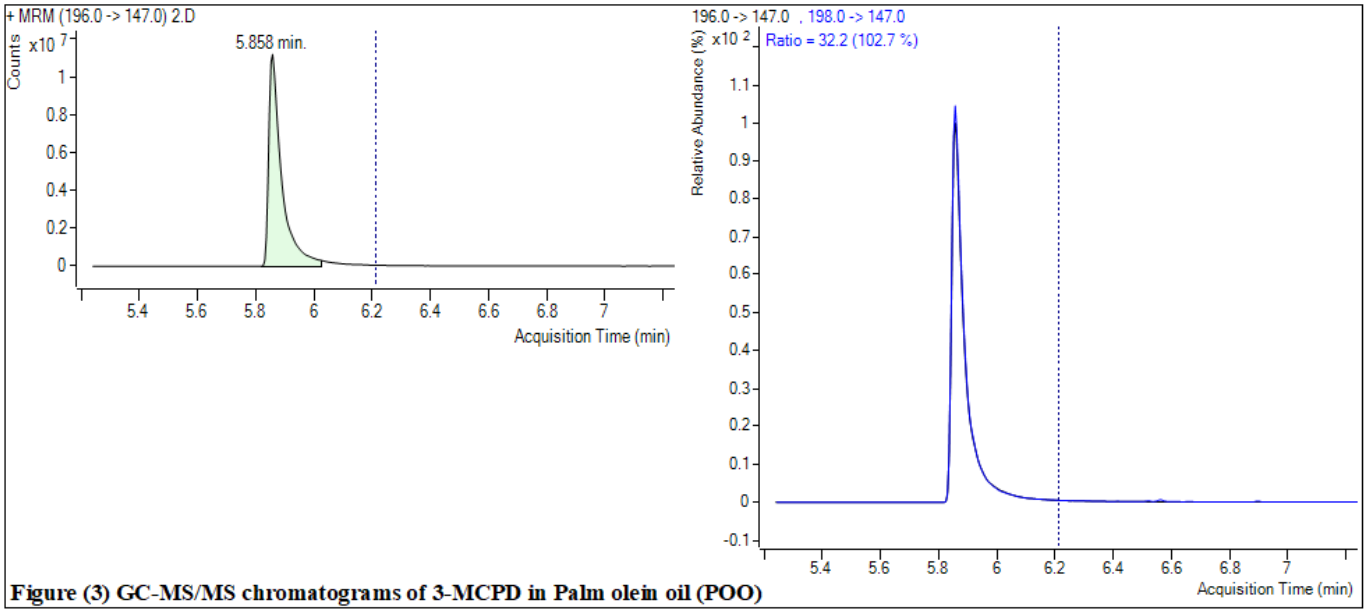


Figure (3) GC-MS/MS chromatograms of 3-MCPD in Palm olein oil (POO)

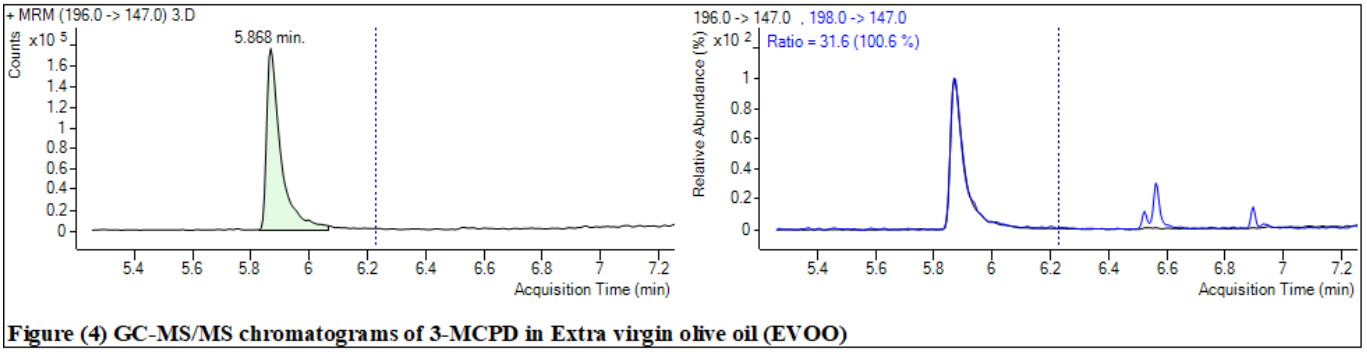


Figure (4) GC-MS/MS chromatograms of 3-MCPD in Extra virgin olive oil (EVOO)

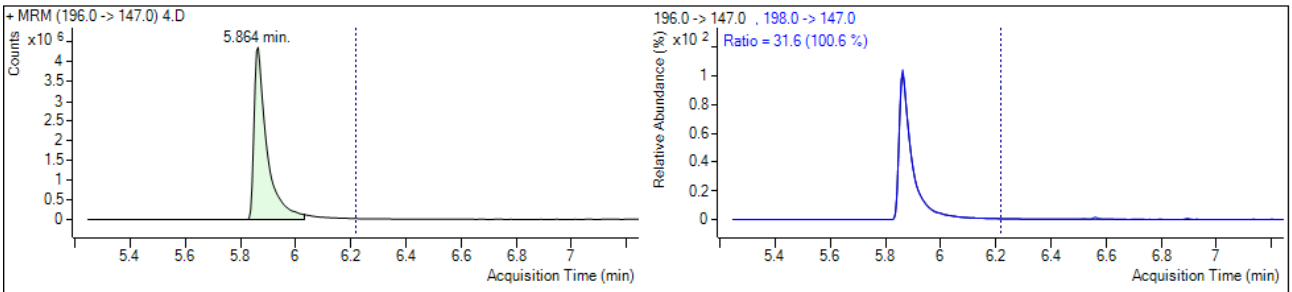


Figure (5) GC-MS/MS chromatograms of 3-MCPD in Corn oil (CO)

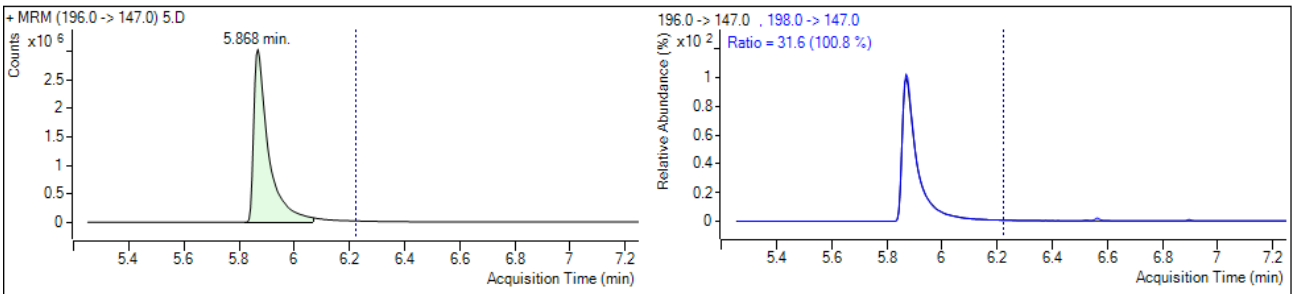


Figure (6) GC-MS/MS chromatograms of 3-MCPD in Sunflower oil (SO)

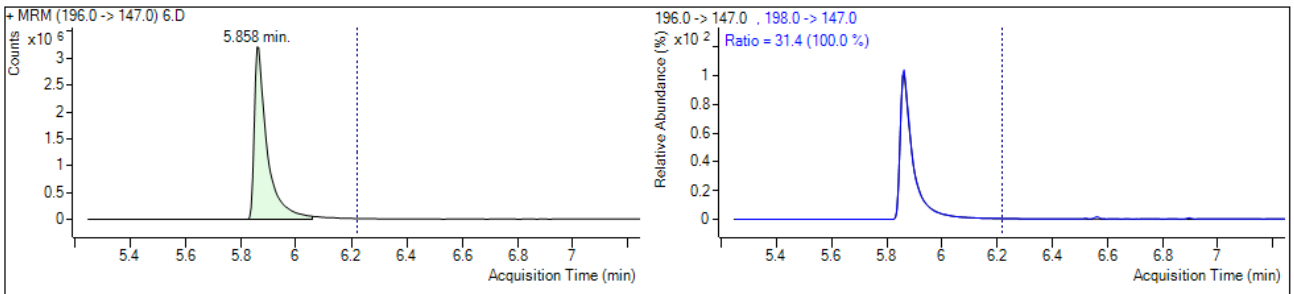


Figure (7) GC-MS/MS chromatograms of 3-MCPD in Soybean oil (SBO)

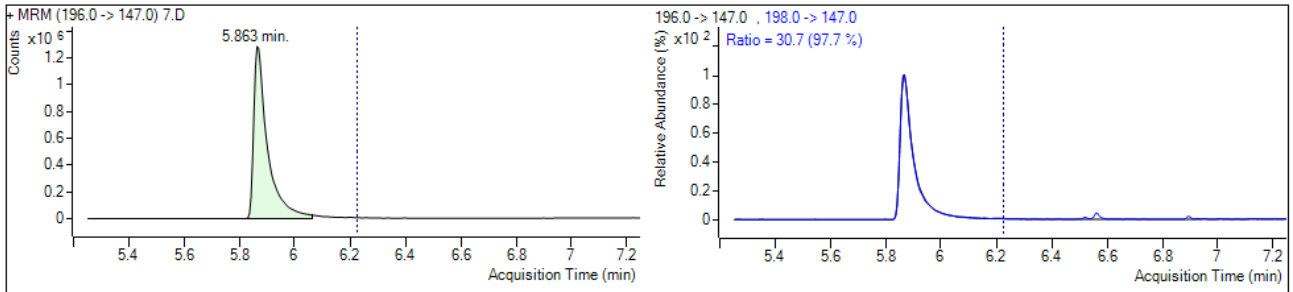


Figure (8) GC-MS/MS chromatograms of 3-MCPD in Olive pomace oil (OPO)

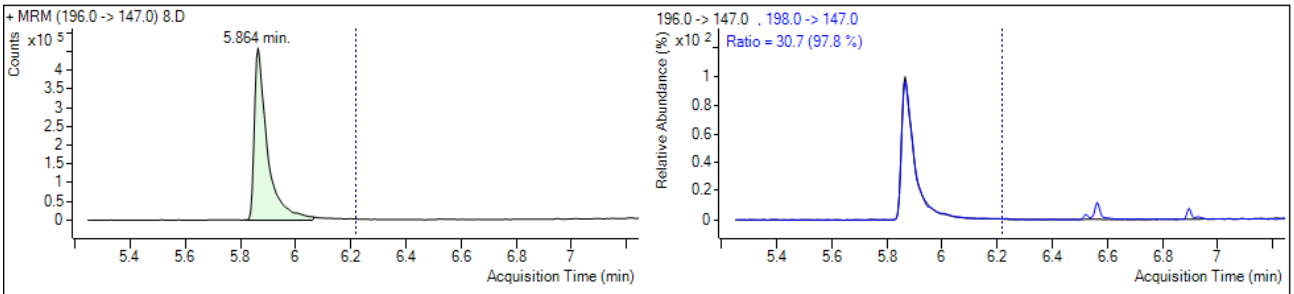
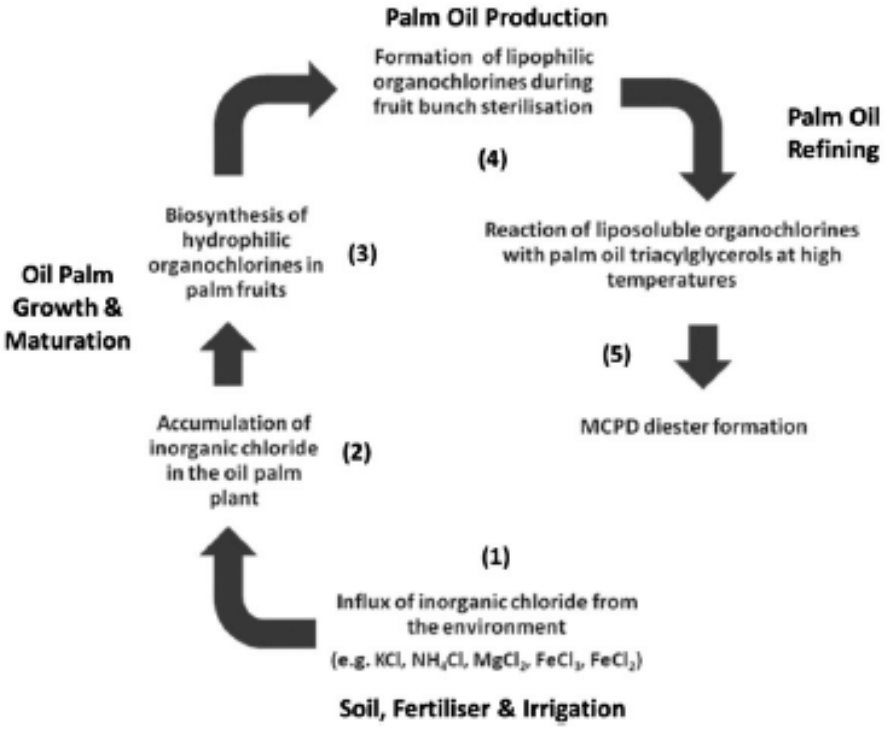


Figure (9) GC-MS/MS chromatograms of 3-MCPD in EVOO+5%SO



Figure(10) Root-cause analysis of the factors involved in the formation of MCPD diesters within the palm oil process chain, including (1) chlorine input into oil palm production, (2) accumulation of inorganic chloride in the plant, (3) bioconversion of inorganic chlorides to organochlorines in the fruits, (4) formation of liposoluble organochlorines during fruit bunch sterilisation and (5) reaction of liposoluble organochlorines with palm oil TAG during oil refining and resulting in MCPD diesters.

Conclusions and Recommendations

Considering the aim of this study to investigate the occurrence and contents of 3-MCPD in some edible oils, and their compliance with standard regulations recommended by Commission Regulation (EU) 2020/1322, higher concentrations of 3-MCPD have been reported for refined edible oils (PO, POO, CO, SO, SBO), compared to unprocessed oil (EVOO) which supports the hypothesis that processing conditions play important roles in the occurrence and formation of 3-MCPD. A recent optimized validated indirect method of determination of 3-MCPD using GC-MS/MS has been verified for fitness-for-use in terms of short sample preparation time, selectivity, sensitivity and accuracy. It is recommended that appropriate efforts to reduce concentrations of 3-MCPD in edible oils continue to be implemented with additional international collaborative studies on refining of edible oils and analysis for 3-MCPD in relevant oil-containing foods to form database for use in future evaluations.

Conflict of Interest

The authors declare no conflict of interest.

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